(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 October 2001 (18.10.2001)

PCT

(10) International Publication Number
WO 01/76371 A1

(51) International Patent Classification[7]: A01N 65/00, 43/90, 27/00 // (A01N 65/00, 25:20, 25:18) (A01N 43/90, 25:20, 25:18) (A01N 27/00, 25:20, 25:18)

(21) International Application Number: PCT/GB01/01572

(22) International Filing Date: 9 April 2001 (09.04.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0008633.0 7 April 2000 (07.04.2000) GB
0024018.4 2 October 2000 (02.10.2000) GB

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
with international search report
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/76371 A1

(54) Title: METHOD OF DEACTIVATING DUST MITE ALLERGENS

(57) Abstract: A method of deactivating a Der-p and/or Der-f allergen which comprises volatilizing into a space to be treated a deactivating amount of a volatile oil selected from cajeput oil (tea tree oil) or an oil comprising one or more terpene hydrocarbons.

METHOD OF DEACTIVATING DUST MITE ALLERGENS

The present invention relates to a method of deactivating dust mite allergens.

Various allergens are known which are transported through the air to trigger a human reaction. For example, it has been known for a long time that house dust can trigger allergenic reactions in humans, such as asthma and rhinitis. It was reported, as early as 1928 that it was the dust mites in the dust that were the primary source of the allergenic response, but it was only in the 1960's that researchers appreciated its significance.

It is believed that the faeces of the house dust mite, *Dermatophogoides farinae* (known as Der-f) and *Dermatophagoides pteronyssinus* (known as Der-p) trigger the immune response of the body, thereby giving rise to well known allergenic symptoms. A review of this is given in Experimental and Applied Acarology, 10 (1991) p. 167-186.

One way to overcome these allergenic responses has been to vacuum clean surfaces, such as carpets, that contain the dust mites and their faeces throughly and often, but that is both time consuming (it has to be regularly done to ensure an allergenic free environment) and is very dependant on the efficiency of the vacuum cleaner and filter bag used, e.g. micron filter bags or two layer vacuum bags.

An alternative method of creating an allergen-free environment has been to denature the allergen, for example, by using an allergen denaturant applied to airborne allergens by means of an aerosol spray device. Such a device produces an aerosol spray when activated and this spray may be targeted at any space which is to be treated.

The allergens to be treated are airborne particles and the use of a known aerosol spray device

results in a low collision rate between the allergen denaturant and the airborne allergens. The practical consequence of such a low collision rate is that the allergen denaturant must be used in a high amount in order to be effective. There may be other consequences such as, in the case where the aerosol spray composition includes a perfume or fragrance, a strong perfume smell or a limited fragrance choice.

PCT/GB98/02863 describes a method for deactivating allergens derived from the Der-f and/or Der-p dust mite species, which comprises contacting the allergen with a deactivating amount of one or more of a variety of 28 deactivants as described. The deactivants which are specified for use include cedarwood oil, hinoki oil and thymol (6-isopropyl-m-cresol).

We have now discovered a group of novel allergen denaturants for the house dust mite Der-p allergen which are derived from natural oils and can be delivered as a vapour to deactivate the allergens.

Accordingly, the present invention provides a method of deactivating a Der-p and/or Der-f allergen which comprises volatilizing into a space to be treated a deactivating amount of a volatile oil selected from cajeput oil (tea tree oil) or an oil comprising one or more terpene hydrocarbons.

Suitable oils comprising one or more terpene hydrocarbons which may be used in the present invention are those which are generically referred to as pinol such as these sold under the names Unitene D® and Unitene LE® (Bush Boake Allen). The main component of both Unitene D and Unitene LE comprise limonene as its major constituent. Unitene D contains significant quantifies of cineole and terpinolene, whilst Unitene LE contains significant quantities of terpene alcohols.

Cajeput oil, which is generally known as tea tree

oil, is obtained from the *Melaleuca leucandra, Melaleuca quinquenervia* or other *Melaleuca* species. The main components of tea tree oil are cineole and terpinene-4-ol.

There are various methods which can be used to volatilise the volatile oils into the air and these delivery methods are discussed below.

The volatile oil may be volatilised by the use of heat to vaporize the oil. For example the volatile oil may be floated on water in an oil burner or heated directly in an oil burner. Alternatively the volatile oil may be vaporized from a heated wick dipped into a reservoir of the volatile oil.

Another method of volatilizing the volatile oil is from an ultra-sonic jet nebuliser which contains water with the volatile oil floated on the surface of the water.

A further method of volatilizing the volatile oil is by the ventilation of a source of the volatile oil using an ion wind. An ion wind generates an ionized air flow which facilitates the evaporation and dispersal of the volatile oil into the air. A unipolar charge is transferred to the molecules of the oil which is evaporated. Optionally the source of the volatile oil may be heated in order to assist evaporation. The ion wind not only facilitates the evaporation and dispersal of the volatile oil but also has the added advantage that the ion wind generating device has no moving parts and thus operates at very low noise levels. The ion wind thus acts as an essentially silent fan. The charged molecules of the vaporized oil are attracted to particles in the air with an opposite or neutral charge and so may be more efficient at denaturing airborne allergens than uncharged molecules. The charged molecules are also attracted to surfaces in the environment which is being treated and thus allergens on surfaces are also

treated.

A method and apparatus for dispersing a volatile composition, such as a volatile oil, is described in our PCT Application No. PCT/GB99/04312.

It will be understood that in order to obtain the desired level of the volatile oil evaporated into a room, the rate of evaporation of the oil will need to be taken into account, the surface area across which the volatile oil is evaporated and the ion wind speed. Higher ion wind speeds will provide faster evaporation of the volatile components and thus the surface area across which the volatile oil is evaporated will need to be adapted to the air flow speed.

The benefit of charging the molecules of the volatile oil using an ion wind is two fold. The individual molecules are attracted as the allergen particles and, since all of the molecules have the same polarity charge, they are repelled one from another. Accordingly, the molecules tend to spread out to a great extent as compared to uncharged molecules.

Allergen particles are normally electrically isolated from their surroundings and will typically be at a potential which is the same as that of their surroundings. An isolated allergenic particle within a cloud of electrically charged molecules is likely to cause distortion of the electrical field so that the attraction of the charged molecules onto the allergen particle will be enhanced.

The volatile oil may be used as such, or may be presented in the form of an emulsion. Generally, the emulsion will be an oil-in-water emulsion comprising up to 5% by weight of the oil. The formation of emulsions is generally well known in the art and is described, for example, in Modern Aspects of Emulsion Science, edited by Bernard P. Binks, The Royal Society of Chemistry, 1998 and Surfactant Science and

Technology, Second Edition, Drew Myers, 1992, VCH Publishers, Inc.

In a still further aspect of the present invention the volatile oil is incorporated into a candle which is burnt in the space to be treated. In carrying out this aspect the present invention the candle which is burnt will generally comprise at least 2% by weight of the volatile oil, preferably at least 5% by weight of the volatile oil and more preferably at least 10% by weight of the volatile oil.

By the term "candle" as used herein is meant a solid, semi-solid or gelled body of a combustible material which contains an axially embedded combustible fibrous wick. When the wick of a candle is lit, the heat so generated melts the combustible material and the resulting liquid flows up the wick by capillary action and is combusted.

Typically, the combustible body of the candle may be a blend of organic materials such as beeswax, paraffin wax, montan wax, carnauba wax, microcrystalline wax, fatty alcohols, fatty acids, fatty esters or natural and synthetic resins. Clear candles may comprise as the combustible material a gel comprising mineral oil containing blends of diblock and triblock copolymers based on synthetic thermoplastic rubbers or a gel obtained by combining a liquid base material of a hydrogenated polyolefin, a gelling agent and optionally a gel enhancing agent.

A wick normally extends longitudinally through the candle body. More than one wick may be used, if desired, but usually a single wick is centrally disposed in the candle body. When a candle wick is ignited, the wick is adapted to burn gradually so that both the wick and the candle body are consumed.

Typically, the weight of candle which is burnt in a particular space to be treated will depend upon the actual volume of the space, e.g. room, to be treated.

An appropriate allergen denaturing effect can be obtained in accordance with the method of the invention by burning in a room of volume 25 to 30m^3 a candle of weight approximately 150g before testing containing 5% by weight of the volatile oil for a period of 5 hours. The amount of the volatile oil which is released from the burning candle can be calculated by weighing the candle at 1 hour intervals.

The length of time for which the candle is burnt in the space to be treated will generally be for up to 2 hours, generally up to 5 hours, although in some circumstances the candle may be burnt for a longer period of time, such as 10 hours or more. However, it will be understood by those skilled in the art that an allergen denaturing effect will be obtained even if the candles containing the selected volatile oils are burnt for a lesser period of time.

The volatile oil may also be delivered by means of a nebuliser in which oil is floated on the surface of water in the nebuliser, or is provided as an oil-in-water emulsion in the nebuliser. The nebuliser comprise a piezo-ceramic element which vibrates in the liquid (at 2-5 MHz) and a plume of liquid is generated by ultrasonic streaming. A dense cloud of very small droplets (<5μm) is then expelled from the surface of the liquid. A fan may be used to assist the expulsion of the nebulised droplets from the vessel.

The present invention will be further described with reference to the following Examples.

Control Pre-treatment Allergen Level

When using house dust for allergen denaturing tests an inherent difficulty is the variability of the amount of allergen in each small sample, even when taken from the same dust reservoir. The amount of dust in the pre-treatment sample must be accurately estimated in order to determine the extent of any

allergen denaturing. In these tests the dust sample was applied to the test exposure surface and then one half of this surface dust was removed to measure the control pre-treatment allergen level of that specific sample. Each control was directly relevant to each sample, which gave the best possible estimate of the level of allergen in the sample before exposure to possible denaturant.

The following Examples all measure the reduction of the house dust mite *(Dermatophagoides pteronyssinus)* allergen - Der p1.

EXAMPLE 1

House dust was passed through a number of sieves and the fraction smaller than 53 micrometres was collected. 0.1g of dust was placed in a small sieve to distribute it evenly over the test surface. The test surface was an aluminium tray 0.6m x 1m. The dust was applied to the tray by moving the sieve continuously over the surface. One half of the dust was then removed by suction onto an in-line filter and the weight recorded, this was the pre-treatment control. The tray was then placed in a plastic lined booth 0.8m x 0.8m x 1.5m. An oil burner containing 800μl of the test sample floated on 6ml of distilled water was placed in the booth, and the booth was sealed. The oil burner candle was lit and allowed to burn until all the liquid had been vaporised (approx. 1 hour). The candle was then smothered and the dust was left exposed in the booth. After 24 hours the tray was removed, the dust was collected from it and its weight recorded. The booth was washed with strong detergent between tests on the same chemical; the booth lining was changed between test chemicals.

Test samples evaluated were:

Hinoki Oil (comparative)

Citronella Oil (comparative)
Tea Tree Oil
Pinol (Unitene D)
Pinol (Unitene LE)

The test samples were assayed for Der p1 using an ELISA (Enzyme linked immunosorbent assay) to determine the allergen content. This was then related to the weight of dust that had been present in each sample. All of the samples were multiplied up to compare the amount of allergen expected to be present in a 0.1g sample of dust. The percentage difference between the control sample and the exposed sample was then obtained and is presented in Figure 1.

The difference in the amount of allergen reduction after exposure to any of the volatile oils released from the oil burner when compared to the inherent loss in sampling was significant when compared in a two-tailed t-test. Therefore, in conditions of the test, exposure to the above oils released from an oil burner resulted in a significant reduction in the allergen contained in the dust samples.

EXAMPLE 2

House dust was passed through a number of sieves and the fraction smaller than 53 micrometres was collected. 0.1g of dust was placed in a small sieve to distribute it evenly over the test surface. The test surface was an aluminium tray 0.6m x 1m. The dust was applied to the tray by moving the sieve continuously over the surface. One half of the dust was then removed by suction onto an in-line filter and the weight recorded, this was the pre-treatment. The tray was then placed in a plastic lined booth 0.8m x 0.8m x 1.5m.

For control tests dust was distributed on the tray, the pre-treatment control collected and the dust

left in the booth for 24 hours. The tray was then removed, the dust was collected from the tray and weighed. In subsequent tests 800μl of volatile oil was added to 150ml of distilled water in the nebuliser. The tests were then completed as in the control tests. The booth was washed with strong detergent between tests. The samples evaluated were:

Tea Tree Oil

Pinol (Unitene D)

Pinol (Unitene LE)

The collected dust samples were assayed for Der p1 using an ELISA to determine the allergen content. This was then related to the weight of dust that had been present in each sample. All of the samples were multiplied up to compare the amount of allergen expected to be present in a 0.1g sample of dust. The percentage difference between the control sample and the exposed sample was then obtained and is presented in Figure 2.

The difference in the amount of allergen reduction after exposure either tea tree oil or Unitene D released from the nebuliser when compared to the loss in sampling control was significant ($P<0.05$) when compared on a two tailed t-test. Therefore, in the conditions of the test, exposure to either tea tree oil or Unitene D released from a nebuliser resulted in a significant reduction in the allergen contained in the dust samples.

EXAMPLE 3

Dust was collected from vacuum cleaner bags and passed through a series of sieves down to 53 microns. Clean petri dishes were labelled with the chemical to be tested and lined with filter paper. 0.3g of dust was added to each dish and spread evenly over the

filter paper. 0.1g of dust was then removed from the filter paper for a control sample. The remaining dust was then redistributed evenly over the filter paper. 2.4g +/- 0.2g of test chemical was sprayed onto the dust sample. The dust sample was left open to the air until the filter paper was dry. The dust was collected into eppendorfs and the weight of dust recovered was measured. 1ml of 1% Bovine Serum Albumin - Phosphate Buffered Saline - Tween (BSA-PBS-T) was added to the control samples. 1ml of 5% BSA-PBS-T was added to the test samples. The samples were left overnight in the fridge and then centrifuged for 5 minutes at 13,000 rpm. The supernatant was pipetted into an eppendorf for assay by Der p1 ELISA.

The test liquids were:

Distilled water
2% Tea Tree Oil in distilled water (Plus 0.1% Tween)
2% Citronella Oil in distilled water (Plus 0.10% Tween)
1% Thymol in distilled water (Plus 0.8% Tween)
2% Hinoki Oil in distilled water (Plus 0.1% Tween)
2% Tannic Acid

5 Replicates were completed for each test liquid. The allergen content of the controls for each replicate was compared with the test sample allergen. The percentage reduction in allergen between the control and the test was determined for each replicate. The average allergen reduction of all 5 replicates is presented in Figure 3.

The water tests showed an average allergen reduction of 34.2%. The addition of Tea Tree Oil to the dust reduced the allergen by another 29.6%, the difference was significant when compared on a t test ($t=4.08$). Thymol reduced the allergen by 23.6% more than the water alone tests, the difference was significant when compared on a t test ($t= 3.3$). The addition of tannic acid to the dust reduced the

allergen by an average of 99.15% in the tests.

When taking the reduction of allergen in the water samples into account, some of the test liquids still significantly reduced the allergen content in the dust samples. Tannic acid was used as a positive control as it is known to denature allergen, and its effect was recorded in the tests. Tea tree oil significantly reduced the allergen content in the dust samples.

EXAMPLE 4

Method

The tests were completed in 28m^3 test rooms with no windows and a door that was closed throughout the duration of the test. The rooms did not contain any furniture and had easily cleaned floors of non-reactive resin. Six test areas 0.7 x 0.7m were marked out on the floor of each room with tape. Each test area was divided into two halves. Test dust had been obtained from household vacuum cleaner bags. House dust was passed through a number of sieves and the fraction smaller than 53 microns was collected. 0.1g of dust was placed in a small sieve to distribute it evenly over the test surface. The dust was applied by moving the sieve continuously over the surface. Dust was removed from half of each of the 6 test areas by suction of 20l/min through an in-line glass fibre filter (2.5cm diameter) and the weight recorded. These were the pre-treatment controls. The selected test candles of approximately 150g before testing were lit and placed in the rooms for 5 hours. The candles were then smothered and the dust was left exposed in the rooms for 16 hours. The dust was then collected as for the controls and weighed.

The collected samples were assayed by Der p1 ELISA to determine the allergen content. This was

then related to the weight of dust that had been present in each sample. All the samples were multiplied up to compare the amount of allergen expected to be present in a 0.1g sample of dust. The percentage differences between the control samples and the exposed samples were then obtained and presented in Figure 4.

During the 5 hour burn period approximately 27g of each of the candles was burnt. For candles B and C detailed below this equated to a release rate of 270µl of essential oil per hour.

Tests completed were:

Test Description

A Unfragranced candle, room relative humidity (rh)

B 5% w/w Tea Tree oil candle, room rh

C 5% w/w Unitene LE candle, room rh

M No Treatment, room rh

The room rh recorded during the tests was between 50 and 57%.

Results

It can be seen from Figure 4 that there is a significant reduction ($P<0.05$) Der p1 allergen content of dust exposed to both the tea tree oil (36.5%) and Unitene LE (30.6%) candle as compared to the no treatment control ($t= 3.19$ and 2.38 respectively).

Discussion

The results indicate that a significant reduction in allergen can be achieved in a room environment by burning candles containing either tea tree oil or Unitene LE for 5 hours.

EXAMPLE 5

Method

British (containing Der p1) or American (containing Der f1) house dust was passed through a number of sieves and the fraction smaller than 53 microns was collected. 0.1g of dust of the selected origin was placed in a small sieve and distributed evenly over the test surface. The test surface was an aluminium tray 0.6m x 1m, which could be easily cleaned with strong detergent. The dust was applied to the tray by moving the sieve continuously over the surface. Half of the dust was then removed by suction of 20L/min through an in-line glass fibre filter (2.5cm diameter) and the weight recorded. This was the pre-treatment control. The tray was then placed in a plastic booth 1 x 0.7m x 0.7m.

The candle to be tested of approximately 150g weight was placed in the booth. The candle was lit and the booth door closed. After approximately 2 hours the temperature and humidity in the booth was measured; the candle was allowed to burn for a total of 5 hours. The candle was then smothered and the dust was left exposed in the booth for 17 hours. The tray was then removed and the booth ventilated. The dust was vacuumed from the tray onto a filter and weighed.

Test candles evaluated were:
Control candle
5% Tea Tree Oil candle
5% Pinol (Unitene LE) candle

Six single exposure replicates were completed for each candle. The collected samples were assayed by Der p1 or Der f1 ELISA to determine the allergen

content. This was then related to the weight of dust that had been present in each sample. All the samples were multiplied up to compare the amount of allergen expected to be present in a 0.1g sample of dust. The percentage difference between the control sample and the exposed sample was then obtained.

The results for Der p1 are presented in Figure 5 and the results for Der f1 are presented in Figure 6.

The reduction of Der p1 allergen concentration in the dust was significant after exposure to either the tea tree oil or Unitene LE candles and the reduction in Der f1 allergen concentration in the dust was significant after exposure to the tea tree oil candle.

EXAMPLE 6

The general procedure of Example 5 was repeated but with three repeated exposures to a candle containing 5% tea tree oil burnt for five hours (i.e. total 15 hours burn) as compared to a single exposure to a candle containing 5% tea tree oil burnt for 5 hours or to a control candle. Six replicate experiments were completed.

The results are given in Figure 7. It will be noted that repeated exposure further reduces the Der p1 allergen concentration of dust on a surface.

EXAMPLE 7

Experiments were completed using the same method as described in Example 5 except that dust samples were exposed in each booth at the same time. 0.025g of dust was distributed evenly over a 0.32m^2 aluminium tray. Half of this was then removed as a control sample as described in Example 5. The tray was placed in the booth. 5 other trays were prepared in this way

and placed in the booth. The 6 trays containing the test dust samples were exposed in the booth to a 5 hour burn of the selected candle. The trays were left exposed in the booth for a further 17 hours, the test dust samples were then collected and assayed by the appropriate ELISA. Figure 8 show a comparison of the % Der pl allergen reduction after exposure to clear gel candles containing 0% (control) or 5% tea tree oil.

The reduction of allergen concentration in the dust was significant after exposure to the gel candle containing tea tree oil.

EXAMPLE 8

Experiments were completed using the same method as described in Example 4. However, instead of burning a candle, a nebuliser was used to deliver the volatile oils.

The ultra-sonic jet nebuliser used in Example 2 was used in these room tests. When the nebuliser was activated a jet of cold, ultra-fine mist was expelled from the top of the reservoir. Tests were completed with 5ml of either tea tree oil or Unitene D floated on top of 150 ml deionised water in the nebuliser.

The nebuliser was activated for 3 hours. It is not known exactly how much of the volatile oil was released as some of the water/oil mixture remained in the nebuliser at the end of the test. Controls were completed with deionised water alone in the nebuliser. The results are given in Figure 9.

There was a significant reduction of the allergen content of the dust after exposure to the tea tree oil or Unitene D.

EXAMPLE 9

Experiments were completed as detailed in Example 1, but with American house dust. Test dust samples were exposed to oil burners in small booths containing 800μl of tea tree oil floated on 6ml of distilled water. These were compared dust lost in sampling. Dust samples were collected after 24 hours and assayed by Der fl ELISA. The results are given in Figure 10.

There was a significant reduction of the allergen content of the dust after exposure to the tea tree oil.

EXAMPLE 10

Experiments were completed using the same method as described in Example 4. However, instead of burning a candle oil burners were used to deliver the tea tree oil.

Two types of oil burners were used in the tests. Small oil burners were used in the small booth tests (detailed in Example 4) and in one of the test room tests. The oil burners were ceramic with a small dish with a 15ml capacity to hold the water and volatile oil. A single tea candle was placed under the suspended dish to evaporate the water and tea tree oil. Large oil burners were used in the remaining tests completed in 28m^3 test rooms. These were also ceramic and had a large dish with a 35ml capacity and were wider in the base so that three tea candles could be placed under the dish to evaporate this larger amount of liquid more efficiently. The tea tree oil was always floated on water in the oil burners as this regulated the temperature and enabled a controlled release rate of the tea tree oil.

Two large oil burners were used in most of the room tests, as this was a much larger volume over

which to deliver the water and tea tree oil. Two large oil burners contained in total 65ml of deionised water and where specified, 5ml of the tea tree oil. This was not a direct translation of the small booth tests as it was found that this would have been unrealistic (336ml water and 44.8ml test chemical). They were placed in the rooms and the candles burnt until all of the liquid had evaporated. Tests were completed with tea tree oil. Controls were conducted with deionised water alone in the oil burners. To quantify any effect due to the candles, tests were conducted with 6 tea candles alone. One test was also completed with a small oil burner containing 6ml of water and 800μl of tea tree oil, so that a comparison could be made with the small booth tests.

The results are given in Figure 11

There was a significant reduction of the allergen content of the dust after exposure to the tea tree oil.

CLAIMS:

1. A method of deactivating a Der-p and/or Der-f allergen which comprises volatilizing into a space to be treated a deactivating amount of a volatile oil selected from cajeput oil (tea tree oil) or an oil comprising one or more terpene hydrocarbons.

2. A method as claimed in claim 1 wherein the volatile oil is heated in order to volatilise it into the air.

3. A method as claimed in claim 1 wherein the volatile oil is volatilised into the air by ventilation of a source of the volatile oil with an ion wind.

4. A method as claimed in claim 1 wherein the volatile oil is volatilized into the air from an ultra-sonic jet nebuliser.

5. A method as claimed in claim 2 or claim 3 wherein the source of volatile oil comprises a wick dipped into a reservoir of the volatile oil.

6. A method as claimed in any one of the preceding claims wherein the volatile oil is provided as a water-in-oil emulsion containing up to 5% by weight of the volatile oil.

7. A method as claimed in claim 1 wherein the volatile oil is incorporated into a candle which is burnt in the space to be treated.

8. A method as claimed in claim 7 wherein the candle which is burnt comprises at least 2% by weight

of the volatile oil.

9. A method as claimed in claim 8 wherein the candle comprises at least 10% by weight of the volatile oil.

10. A method as claimed in any one of claims 7 to 9 wherein the candle is burnt for 2 hours or more.

11. A method as claimed in any one of the preceding claims wherein the oil comprising one or more terpene hydrocarbons is a pinol.

Fig.1.

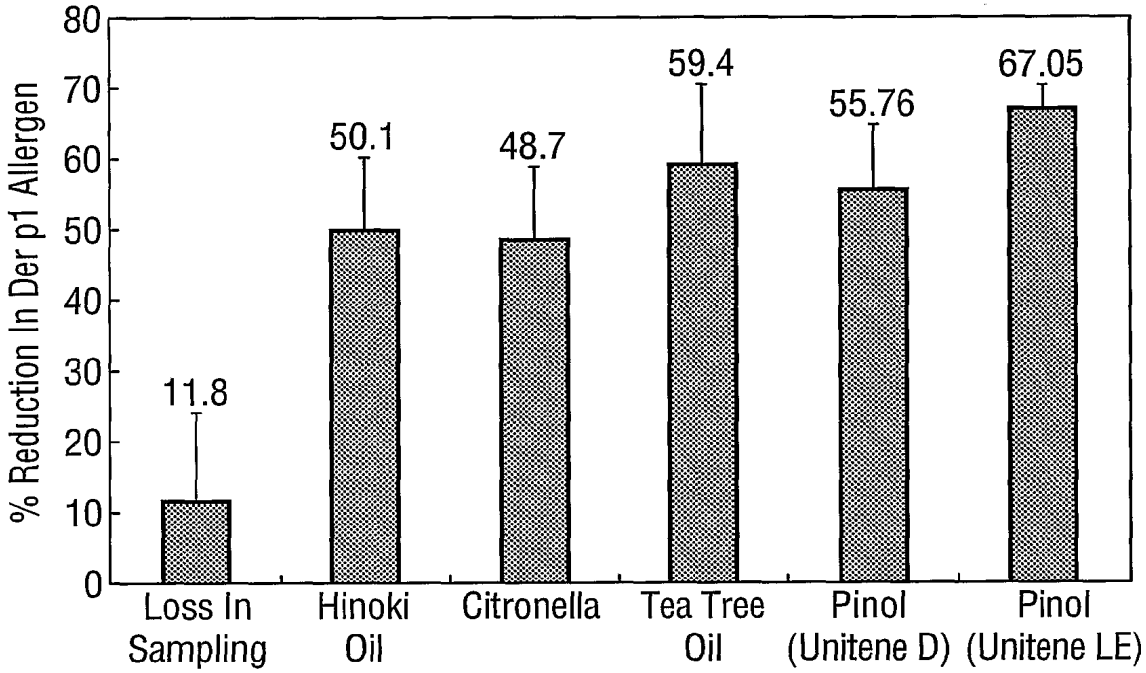

% Reduction In Der p1 Allergen
80
70
60
50
40
30
20
10
0
11.8
50.1
48.7
59.4
55.76
67.05
Loss In Sampling
Hinoki Oil
Citronella
Tea Tree Oil
Pinol (Unitene D)
Pinol (Unitene LE)

Fig.2.

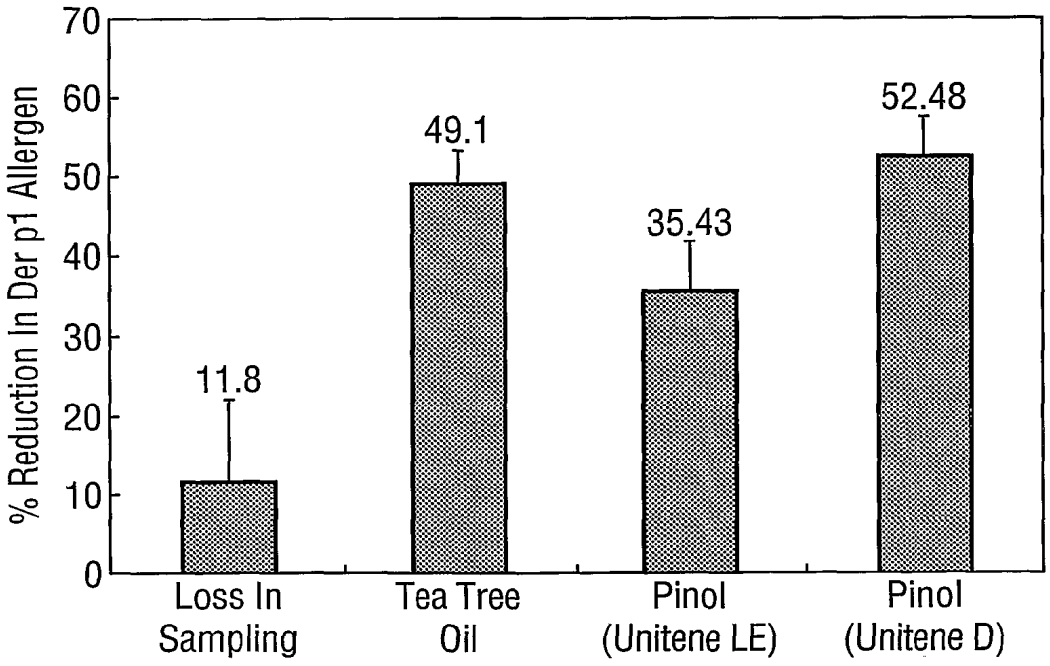

% Reduction In Der p1 Allergen
70
60
50
40
30
20
10
0
11.8
49.1
35.43
52.48
Loss In Sampling
Tea Tree Oil
Pinol (Unitene LE)
Pinol (Unitene D)

Fig.3.

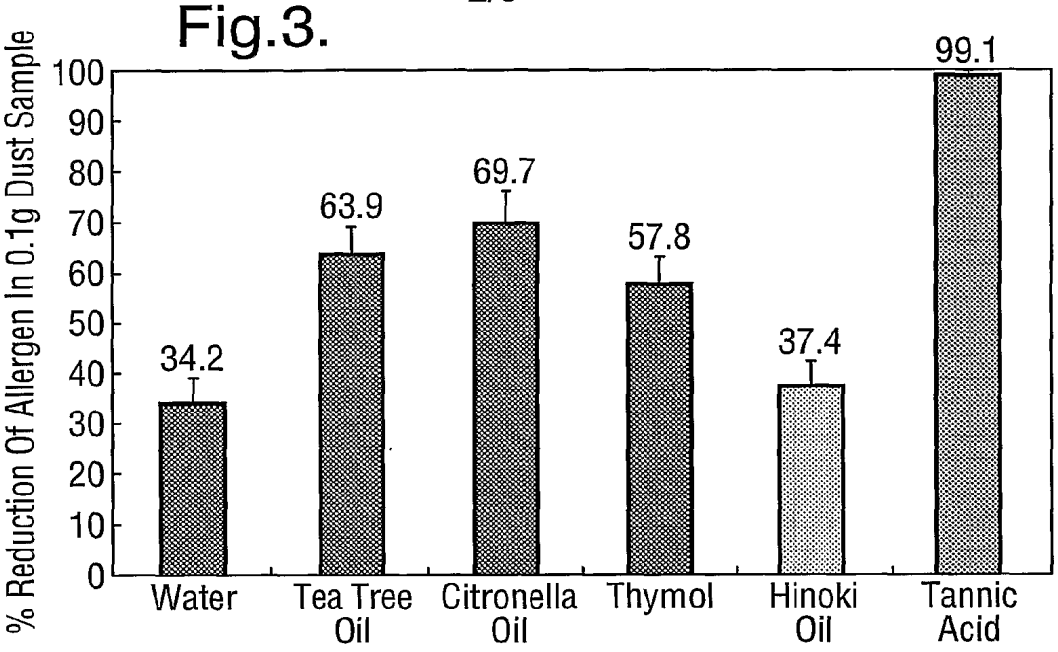

% Reduction Of Allergen In 0.1g Dust Sample
100
90
80
70
60
50
40
30
20
10
0
34.2
63.9
69.7
57.8
37.4
99.1
Water
Tea Tree Oil
Citronella Oil
Thymol
Hinoki Oil
Tannic Acid

Fig.4.

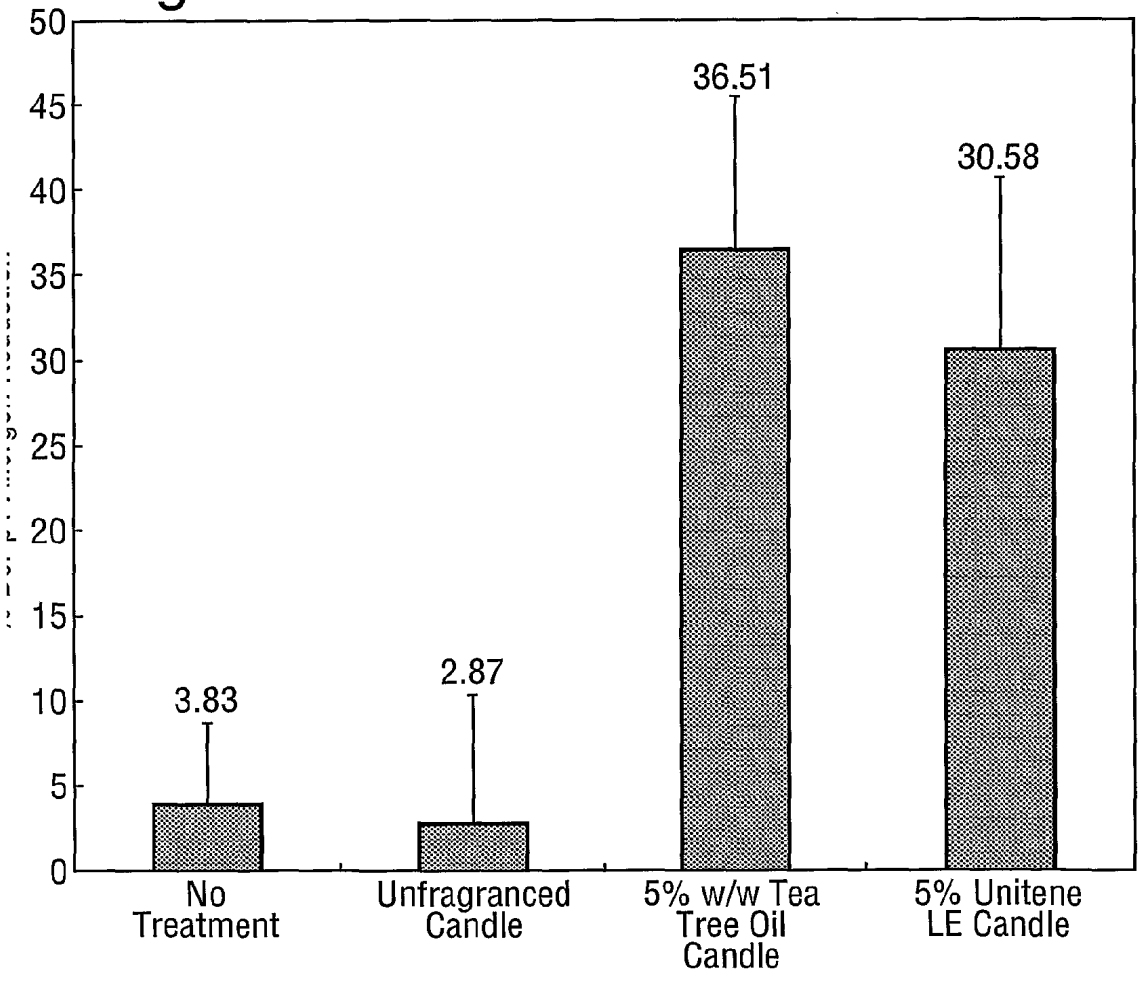

50
45
40
35
30
25
20
15
10
5
0
3.83
2.87
36.51
30.58
No Treatment
Unfragranced Candle
5% w/w Tea Tree Oil Candle
5% Unitene LE Candle

Fig.5.

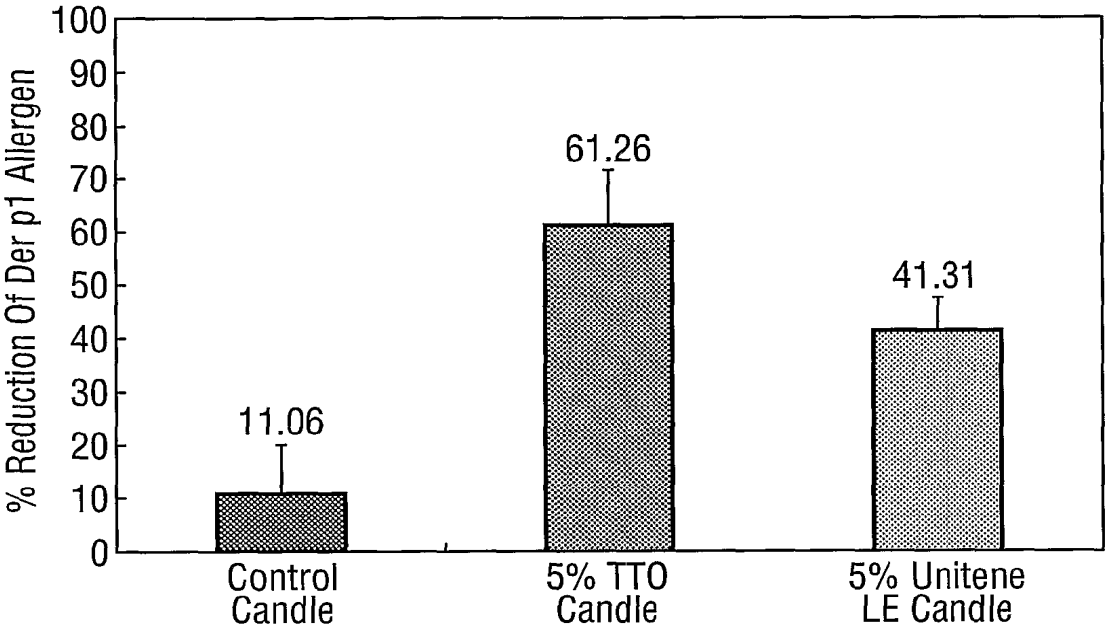

% Reduction Of Der p1 Allergen
100
90
80
70
60
50
40
30
20
10
0
11.06
61.26
41.31
Control Candle
5% TTO Candle
5% Unitene LE Candle

Fig.6.

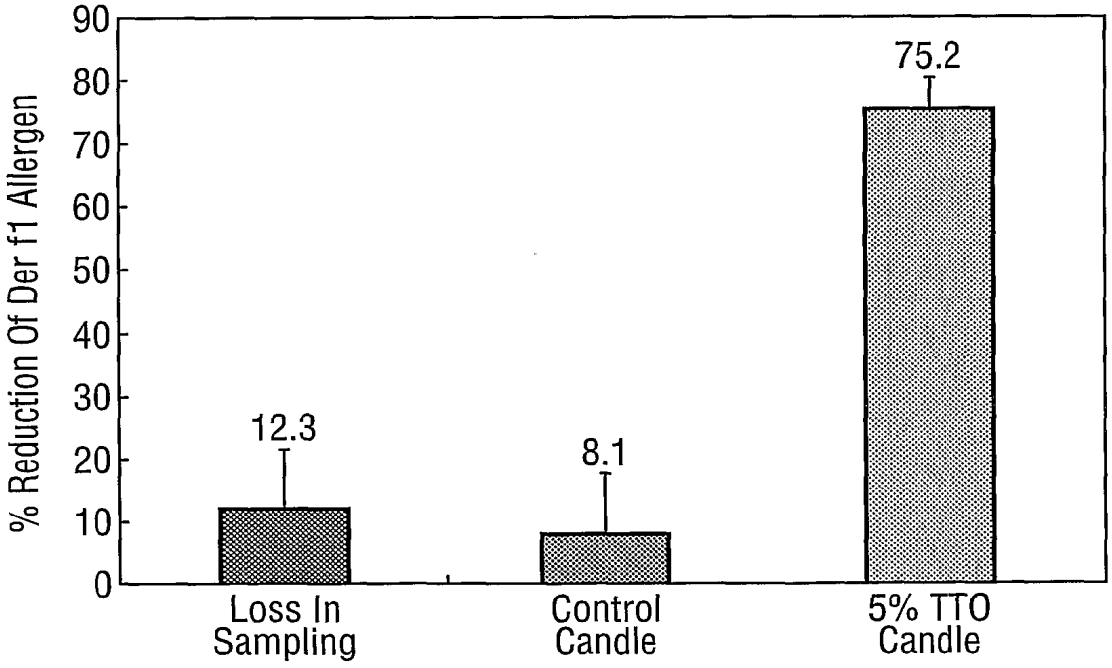

% Reduction Of Der f1 Allergen
90
80
70
60
50
40
30
20
10
0
12.3
8.1
75.2
Loss In Sampling
Control Candle
5% TTO Candle

Fig.7.

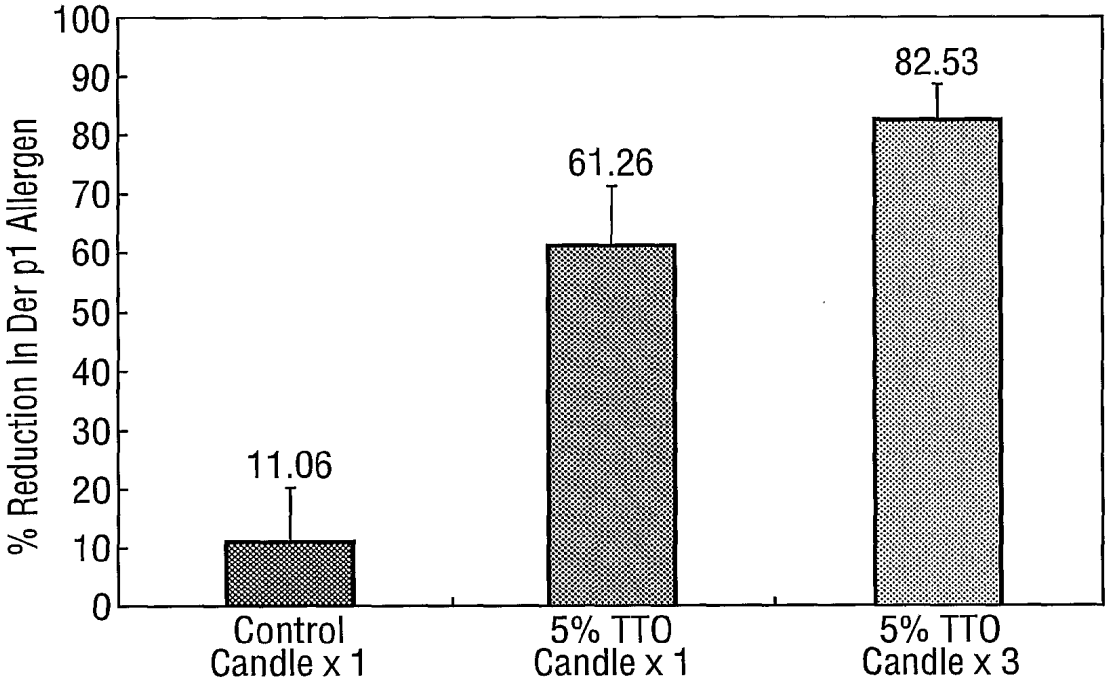

% Reduction In Der p1 Allergen
100
90
80
70
60
50
40
30
20
10
0
11.06
61.26
82.53
Control Candle x 1
5% TTO Candle x 1
5% TTO Candle x 3

Fig.8.

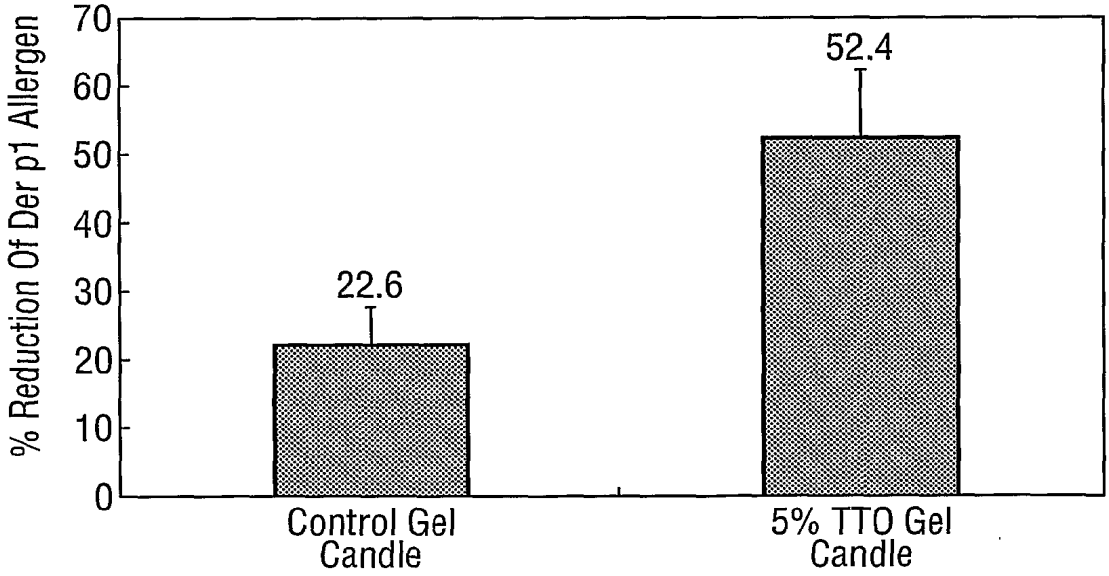

% Reduction Of Der p1 Allergen
70
60
50
40
30
20
10
0
22.6
52.4
Control Gel Candle
5% TTO Gel Candle

Fig.9.

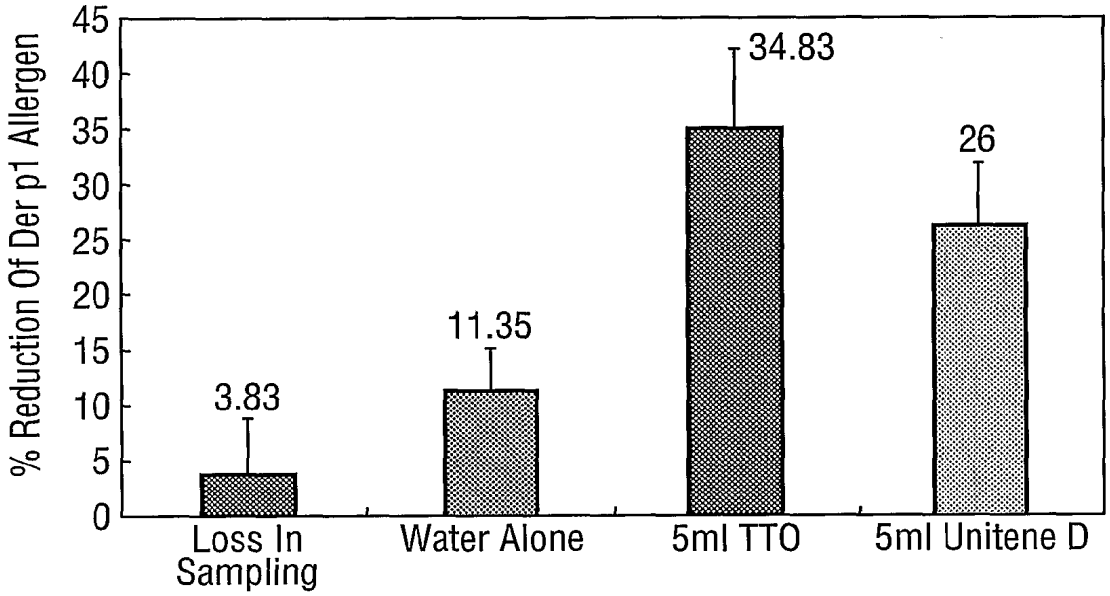

% Reduction Of Der p1 Allergen
45
40
35
30
25
20
15
10
5
0
3.83
11.35
34.83
26
Loss In Sampling
Water Alone
5ml TTO
5ml Unitene D

Fig.10.

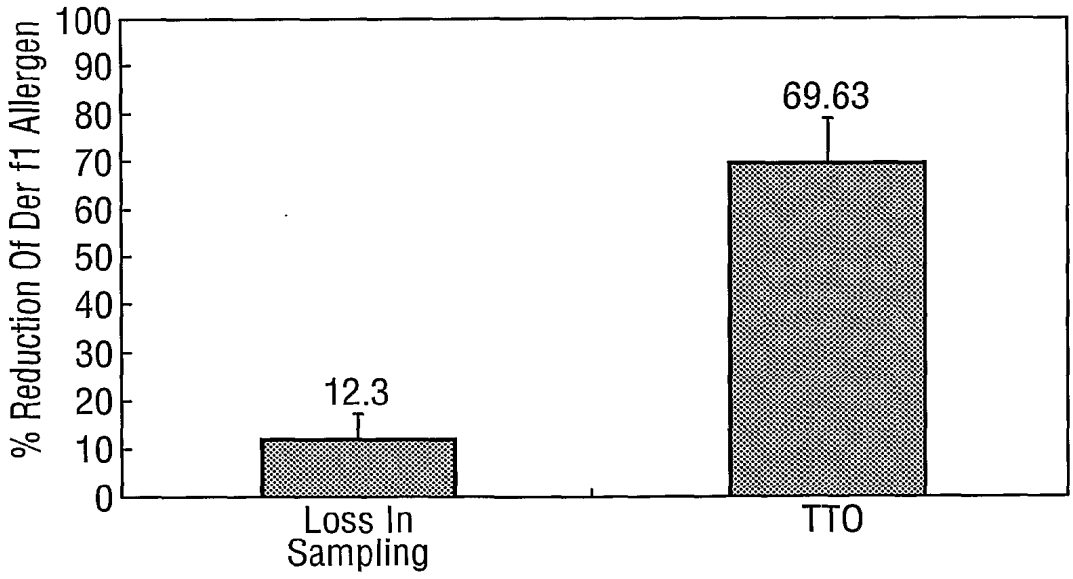
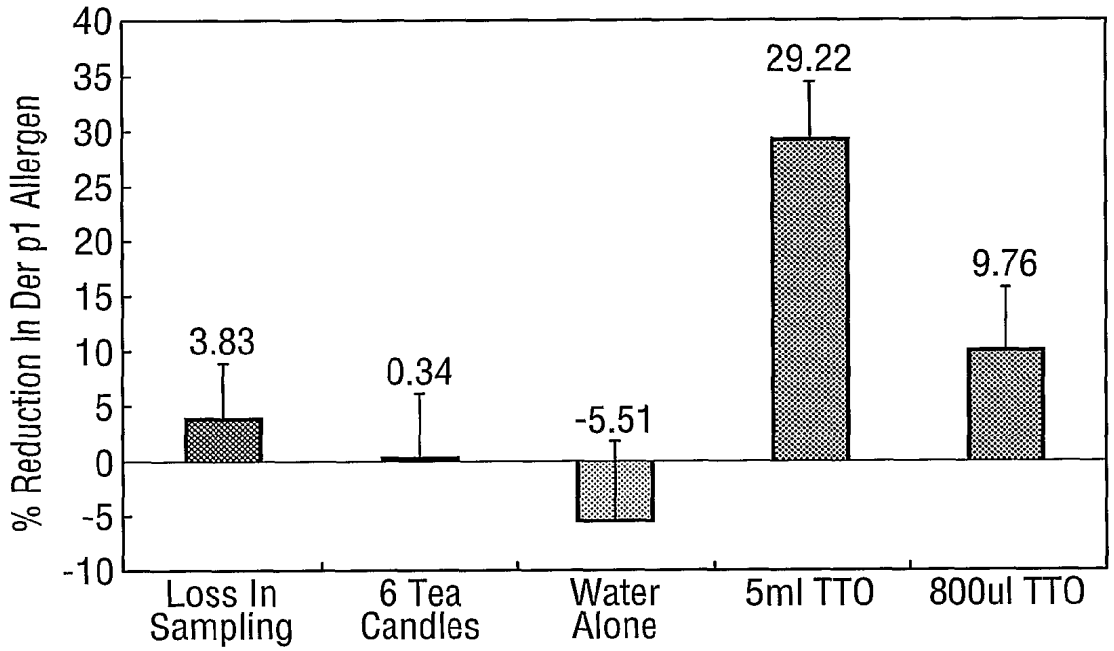

% Reduction Of Der f1 Allergen
100
90
80
70
60
50
40
30
20
10
0
12.3
69.63
Loss In Sampling
TTO

Fig.11.


% Reduction In Der p1 Allergen
40
35
30
25
20
15
10
5
0
-5
-10
3.83
0.34
-5.51
29.22
9.76
Loss In Sampling
6 Tea Candles
Water Alone
5ml TTO
800ul TTO

INTERNATIONAL SEARCH REPORT

Interr al Application No
PCT/GB 01/01572

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N65/00 A01N43/90 A01N27/00 //(A01N65/00,25:20,25:18), (A01N43/90,25:20,25:18),(A01N27/00,25:20,25:18)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, CHEM ABS Data, CAB Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
31 August 2001	10/09/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Lamers, W

INTERNATIONAL SEARCH REPORT

Intern I Application No
PCT/GB 01/01572

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 99 15208 A (THOMPSON IAN ANDREW ;MCKECHNIE MALCOLM TOM (GB); CORNELIUS GAY (GB) 1 April 1999 (1999-04-01) page 2, line 27 -page 6, line 20	1-11
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